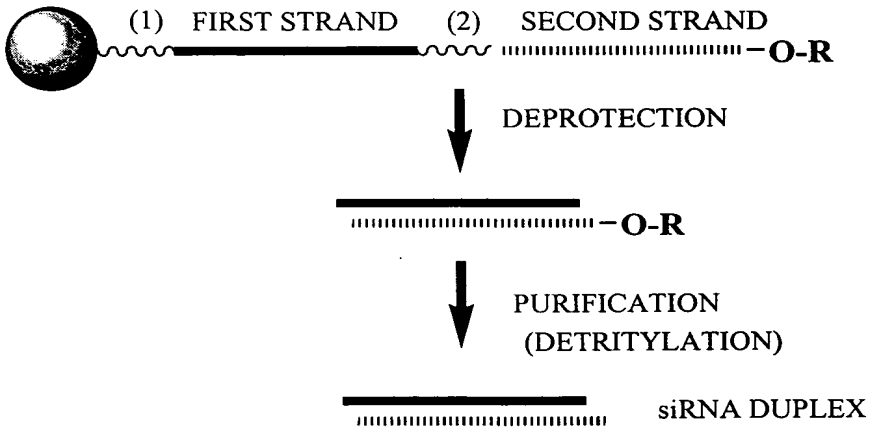
Figure 1

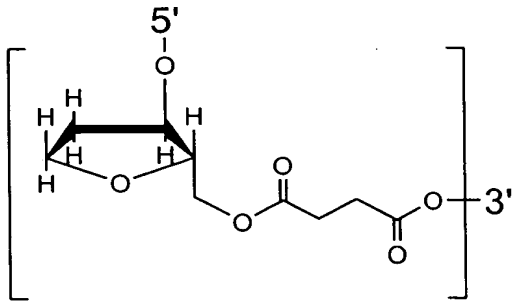


= SOLID SUPPORT

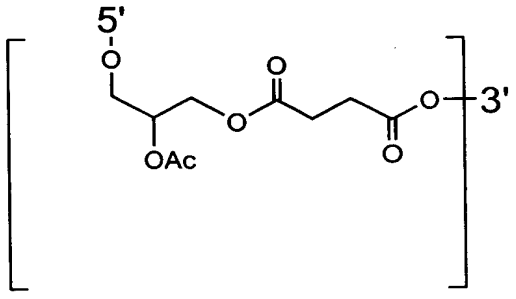
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

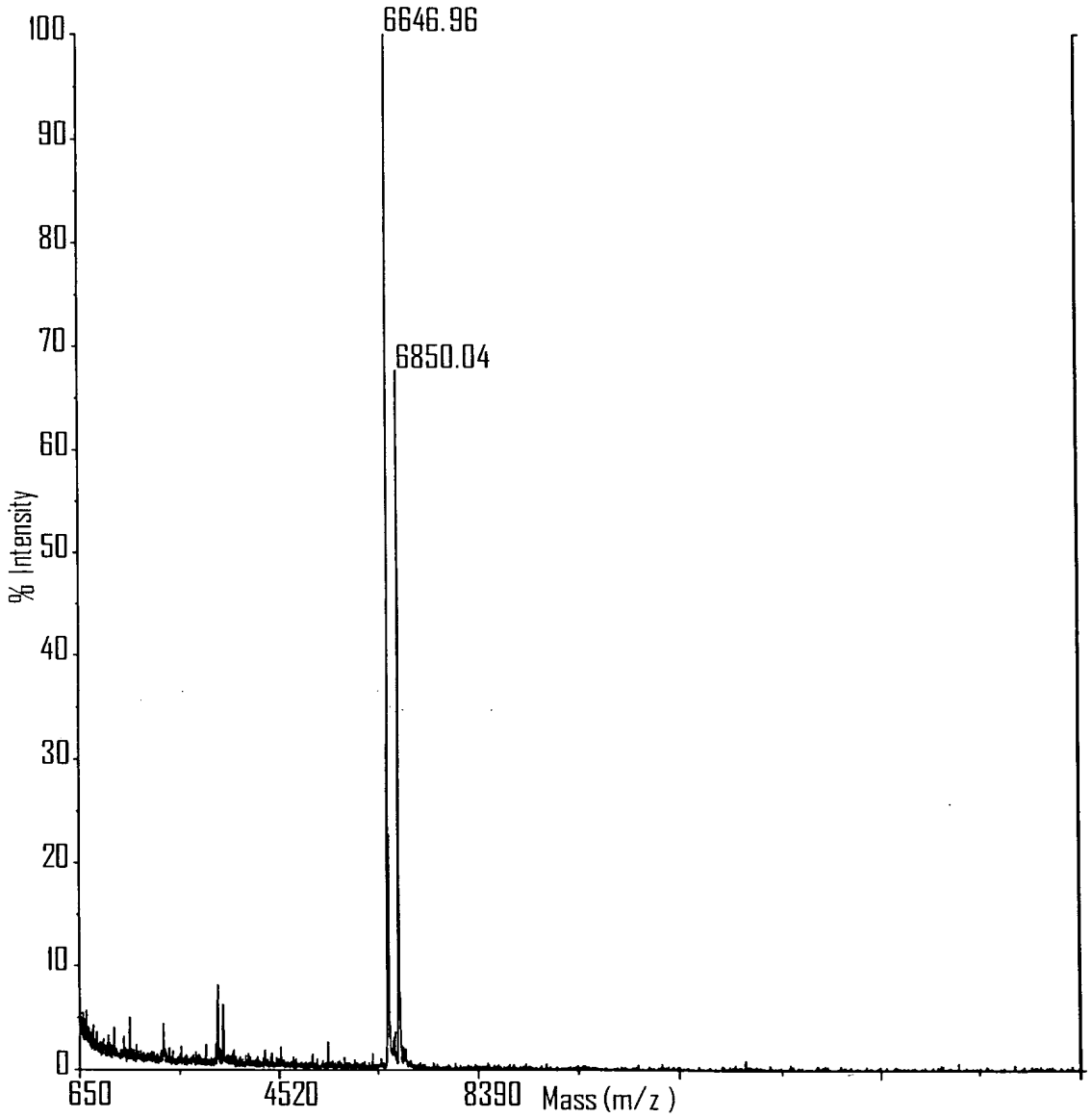

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

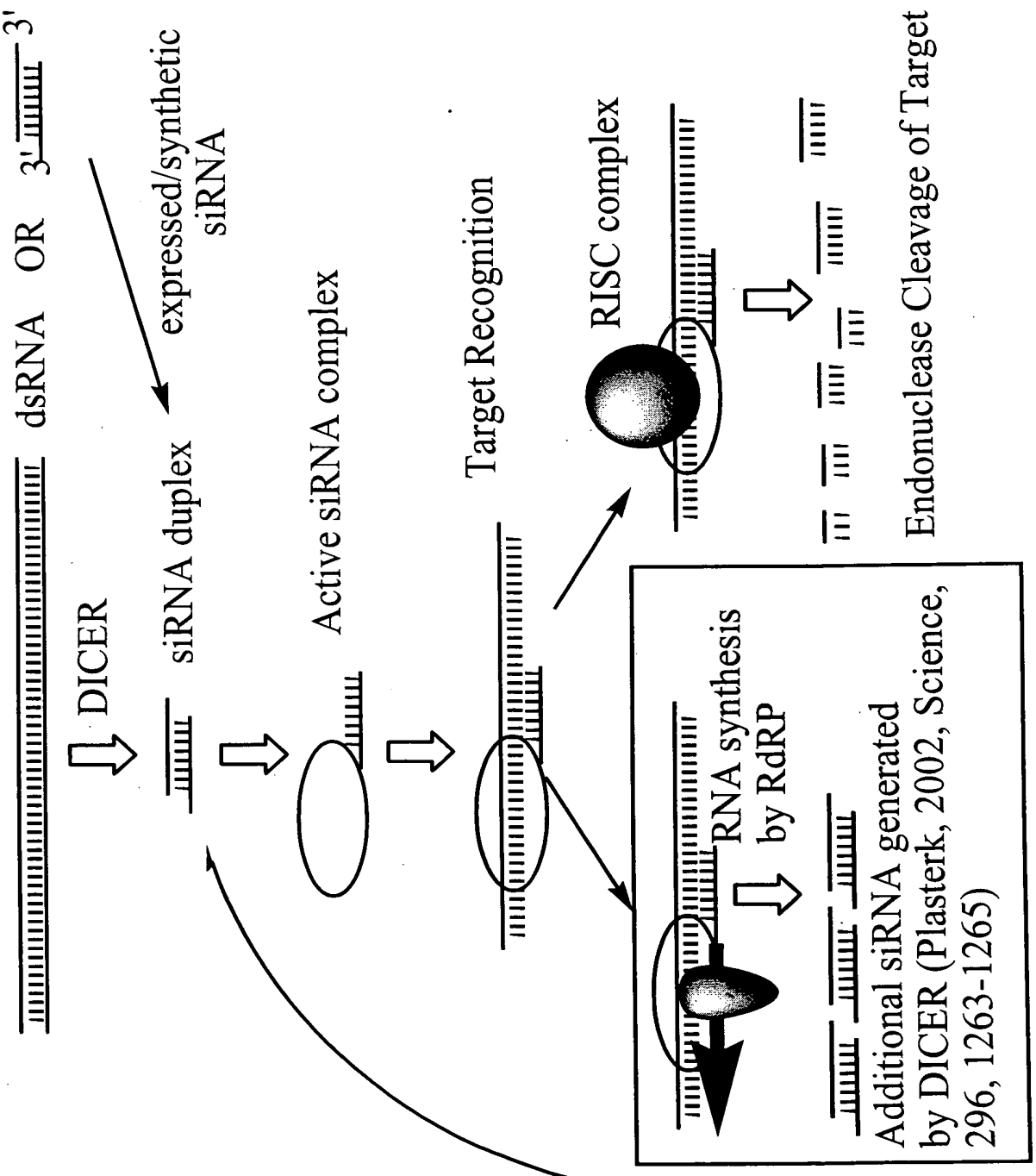

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 287)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 288)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 289)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_sN) -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 290)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 291)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 292)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 293)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 290)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 294)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 290)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 293)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'
3'- L-(N_sN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 295)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5

A

SENSE STRAND (SEQ ID NO 296)

5'- iB- G A A C A A C A A U G A U G C U G C U *T T*-iB -3'

3'- L-T_S*T* C U U G U U G U U A C U A C G A C G A -5'

ANTISENSE STRAND (SEQ ID NO 297)

B

SENSE STRAND (SEQ ID NO 298)

5'- g a a c a a c a a u g a u g c u g c u T_S*T* -3'

3'- L-T_S*T c u u g u u g u u a c u a c g a c g a* -5'

ANTISENSE STRAND (SEQ ID NO 299)

C

SENSE STRAND (SEQ ID NO 300)

5'- iB-G A A c A A c A A u G A u G c u G c u *T T*-iB -3'

3'- L-T_S*T c u u* G *u u* G *u u* A *c u* A *c* G A *c* G A -5'

ANTISENSE STRAND (SEQ ID NO 301)

D

SENSE STRAND (SEQ ID NO 302)

5'- iB-*G A A c A A c A A u G A u G c u G c u T T*-iB -3'

3'- L-T_S*T c u u g u u g u u a c u a c g a c g a* -5'

ANTISENSE STRAND (SEQ ID NO 299)

E

SENSE STRAND (SEQ ID NO 303)

5'- iB-G A A *c* A A *c* A A *u* G A *u* G *c u* G *c u T T*-iB -3'

3'- L-T_S*T c u u g u u g u u a c u a c g a c g a* -5'

ANTISENSE STRAND (SEQ ID NO 299)

F

SENSE STRAND (SEQ ID NO 302)

5'- iB-*G A A c A A c A A u G A u G c u G c u T T*-iB -3'

3'- L-T_S*T c u u* G *u u* G *u u A c u A c G A c G A* -5'

ANTISENSE STRAND (SEQ ID NO 304)

lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY

iB = INVERTED DEOXYABASIC

L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT

S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

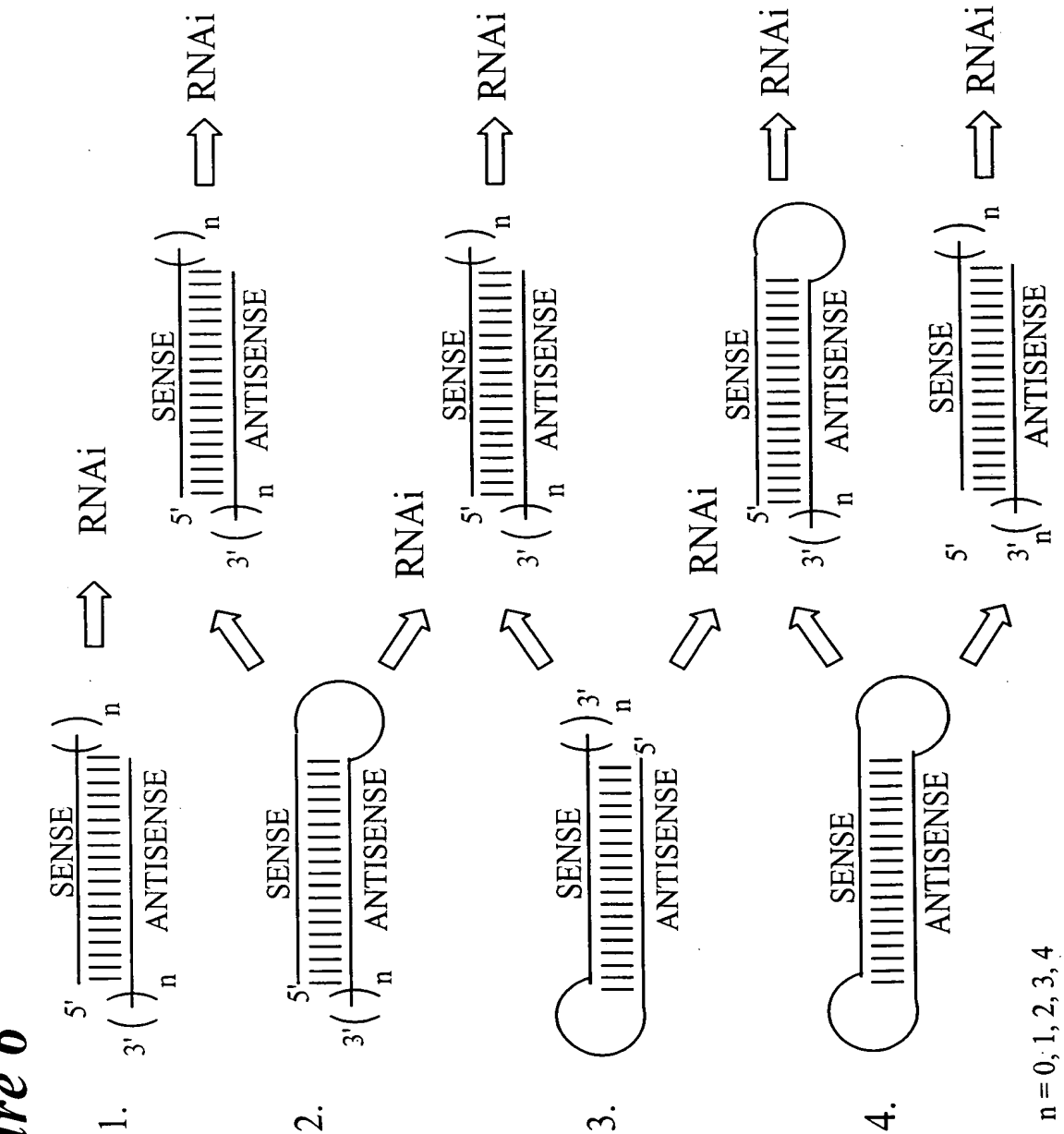


Figure 7

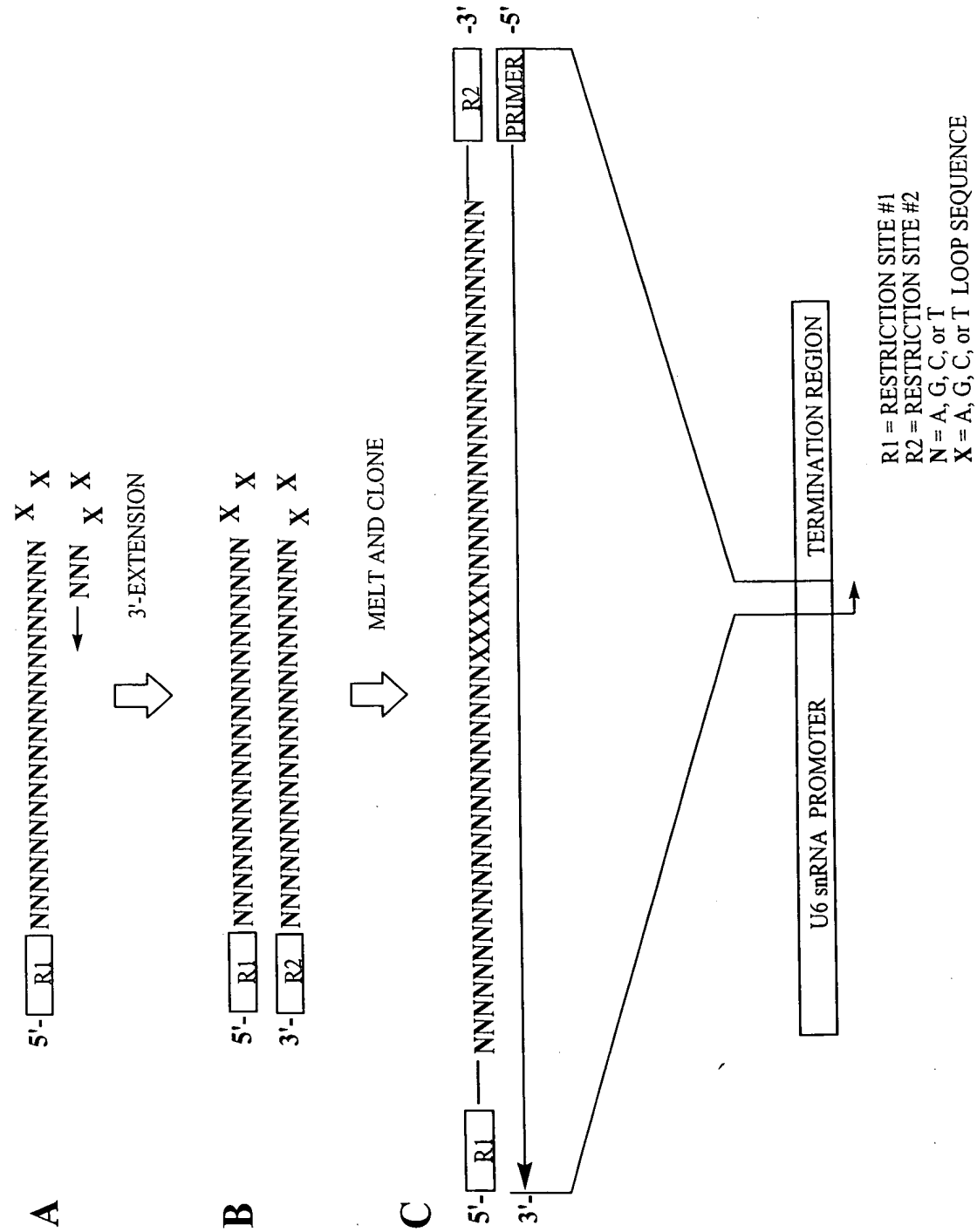

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 — NNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNN — R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8

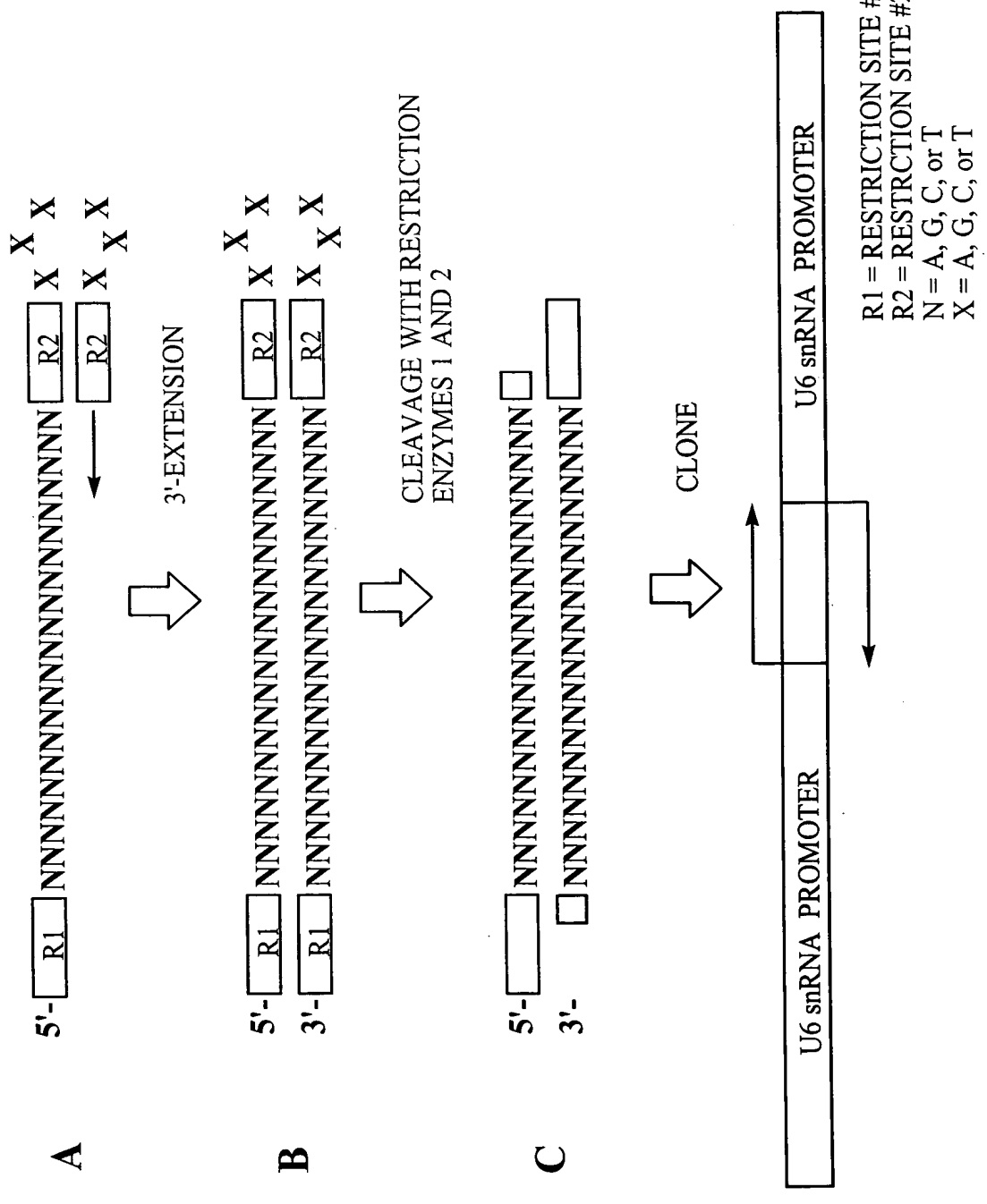

A
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
R2 X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
3'- R1 NNNNNNNNNNNNNNNNNNN R2 X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'- NNNNNNNNNNNNNNNNNNN
3'- NNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRCTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

Figure 9: Target site Selection using siRNA

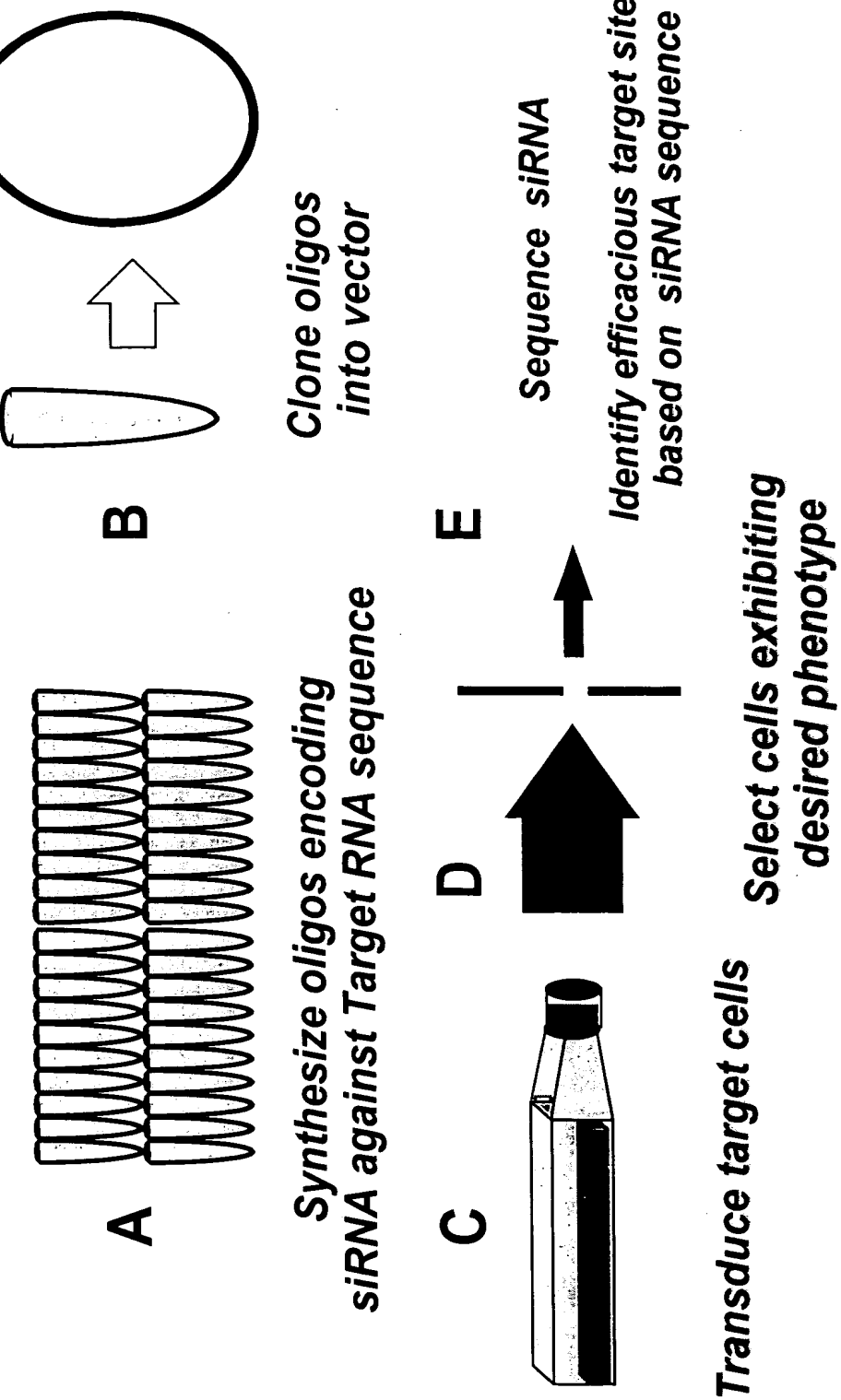
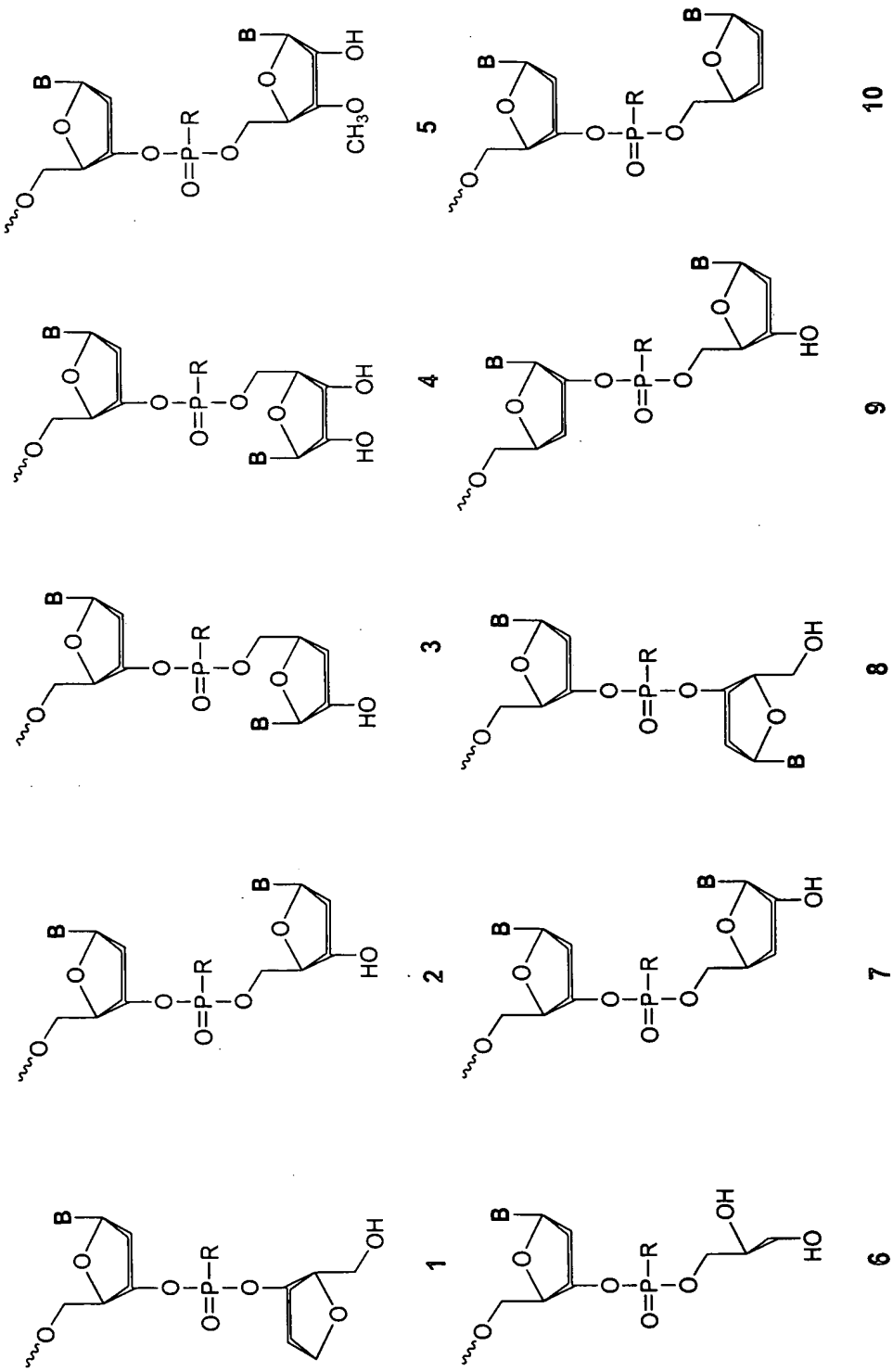


Figure 10



R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

Figure 11: Modification Strategy

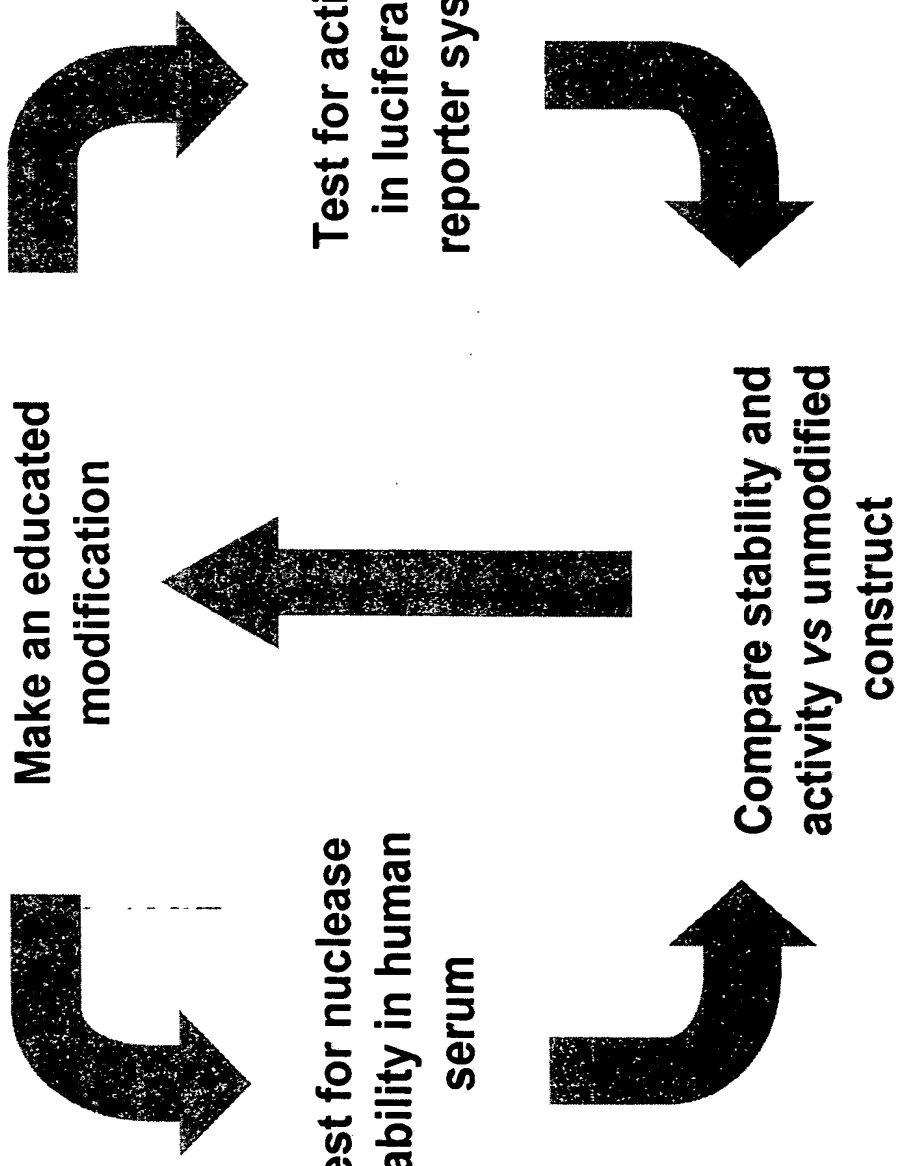
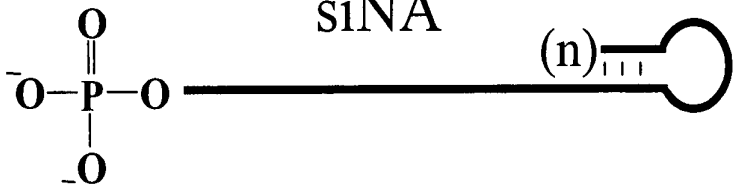


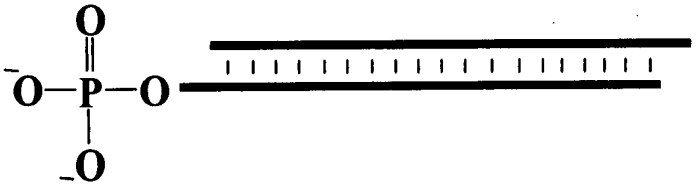
Figure 12: Phosphorylated siNA constructs

Phosphates can be modified as described herein

Asymmetric hairpin siNA



Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 13: 5'-phosphate modifications

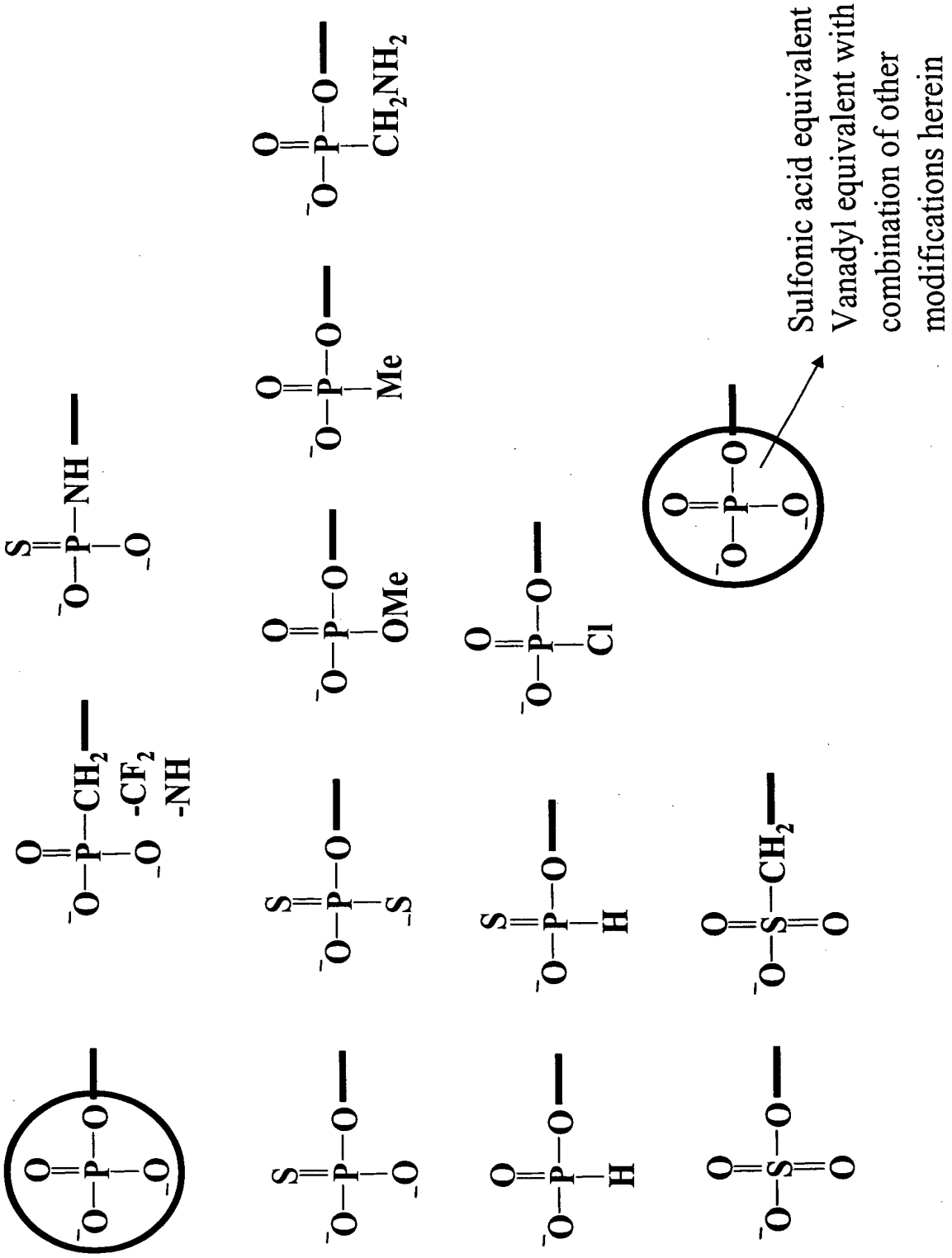


Figure 14A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences

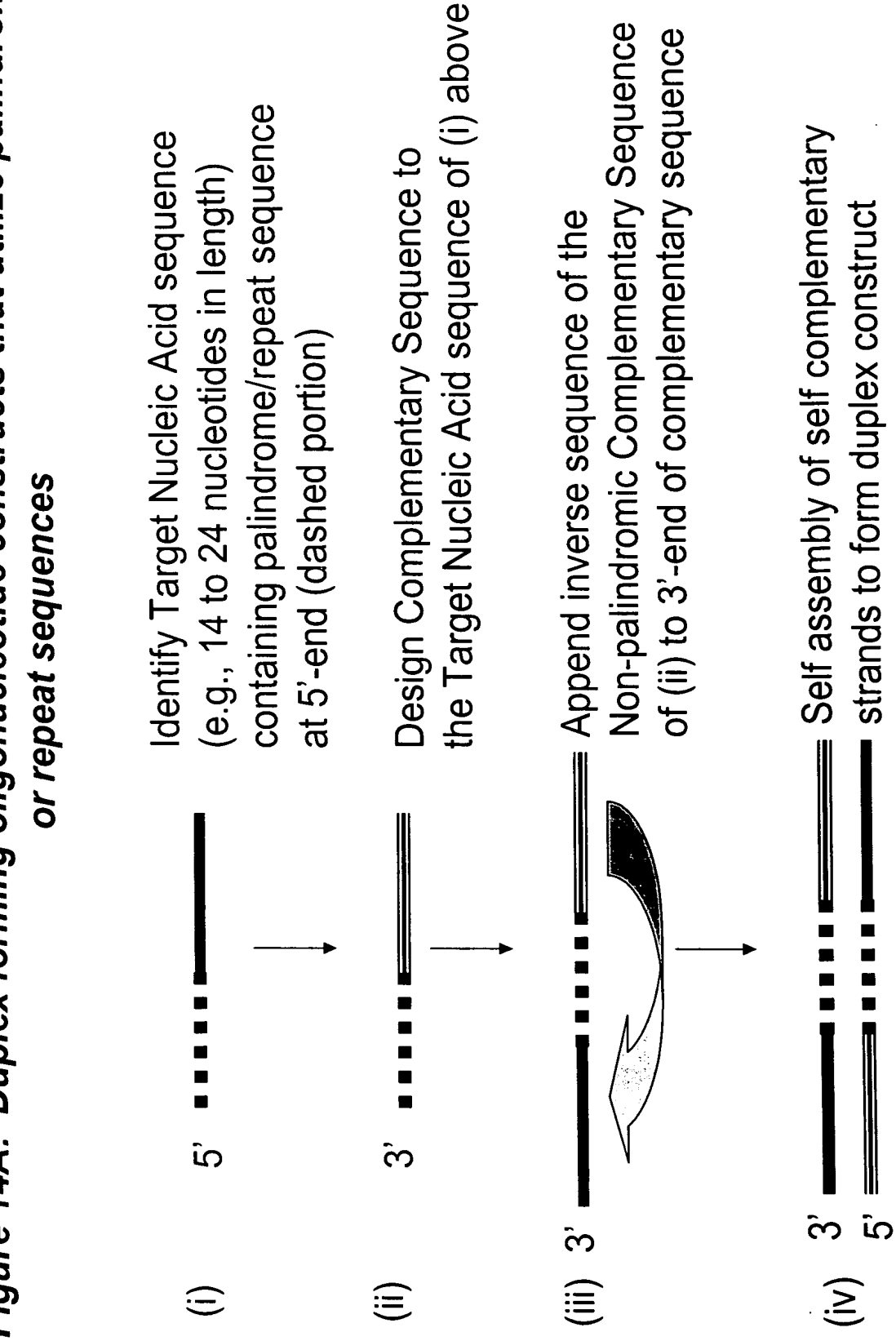


Figure 14B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence

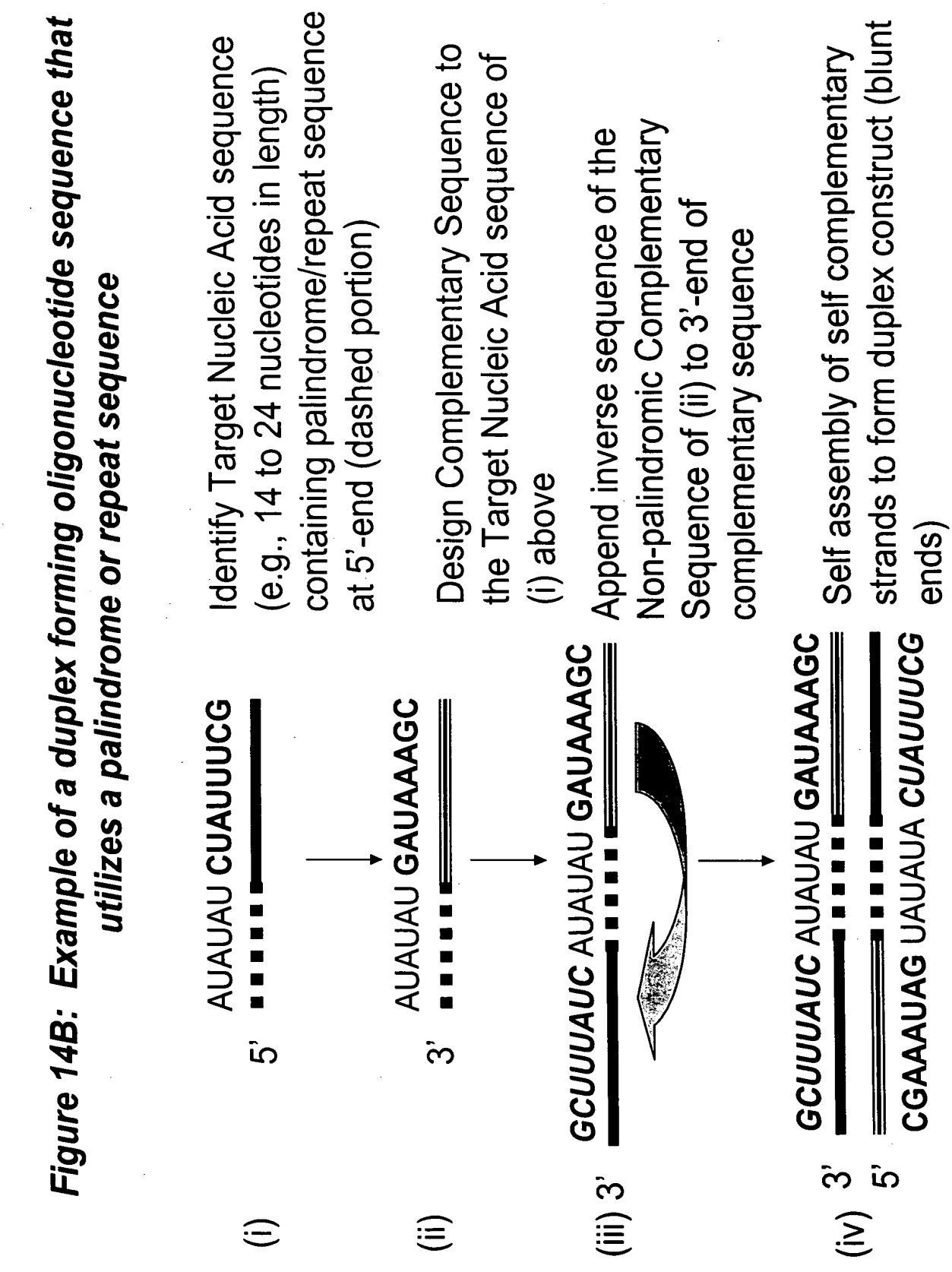


Figure 14C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

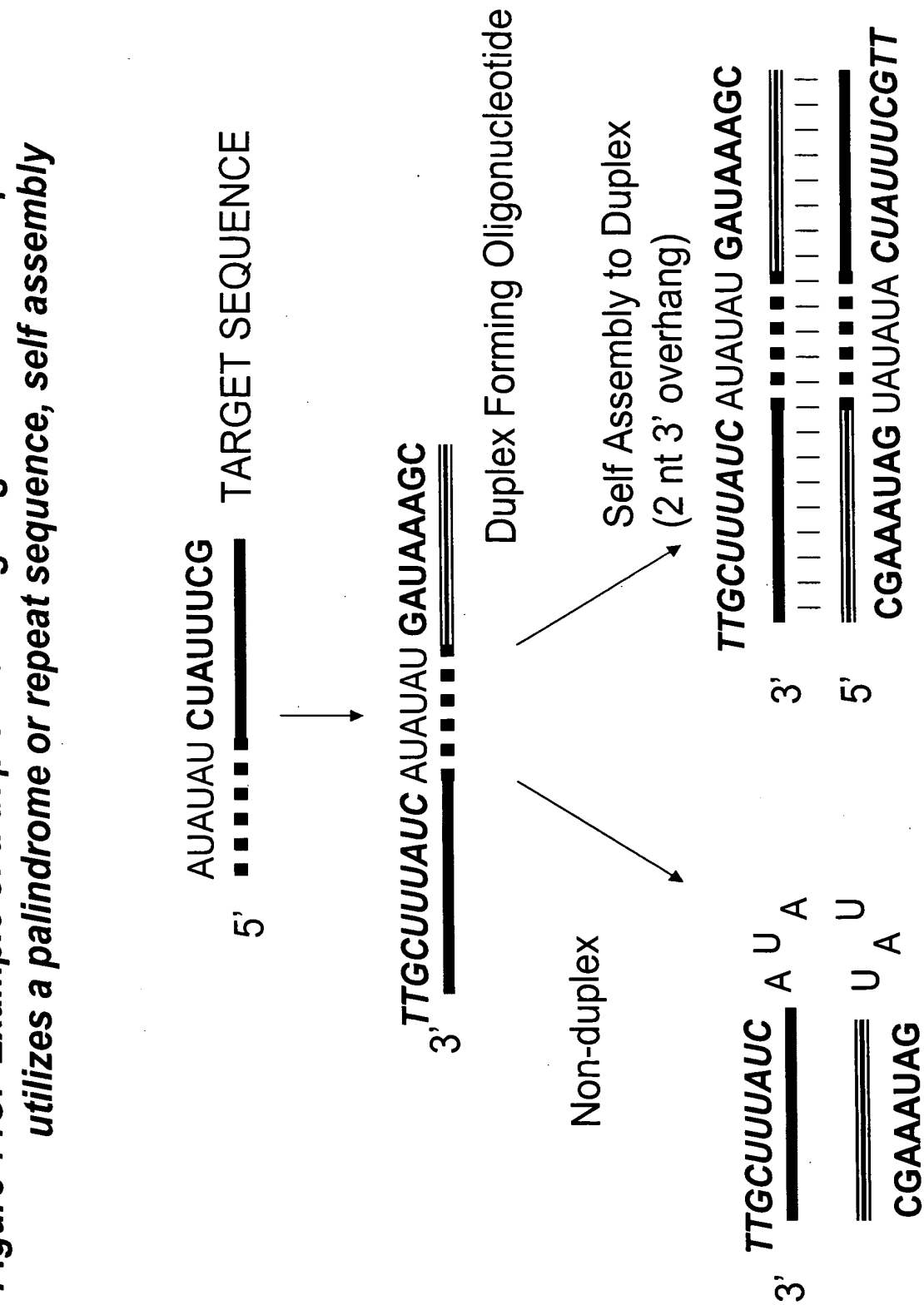


Figure 14D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

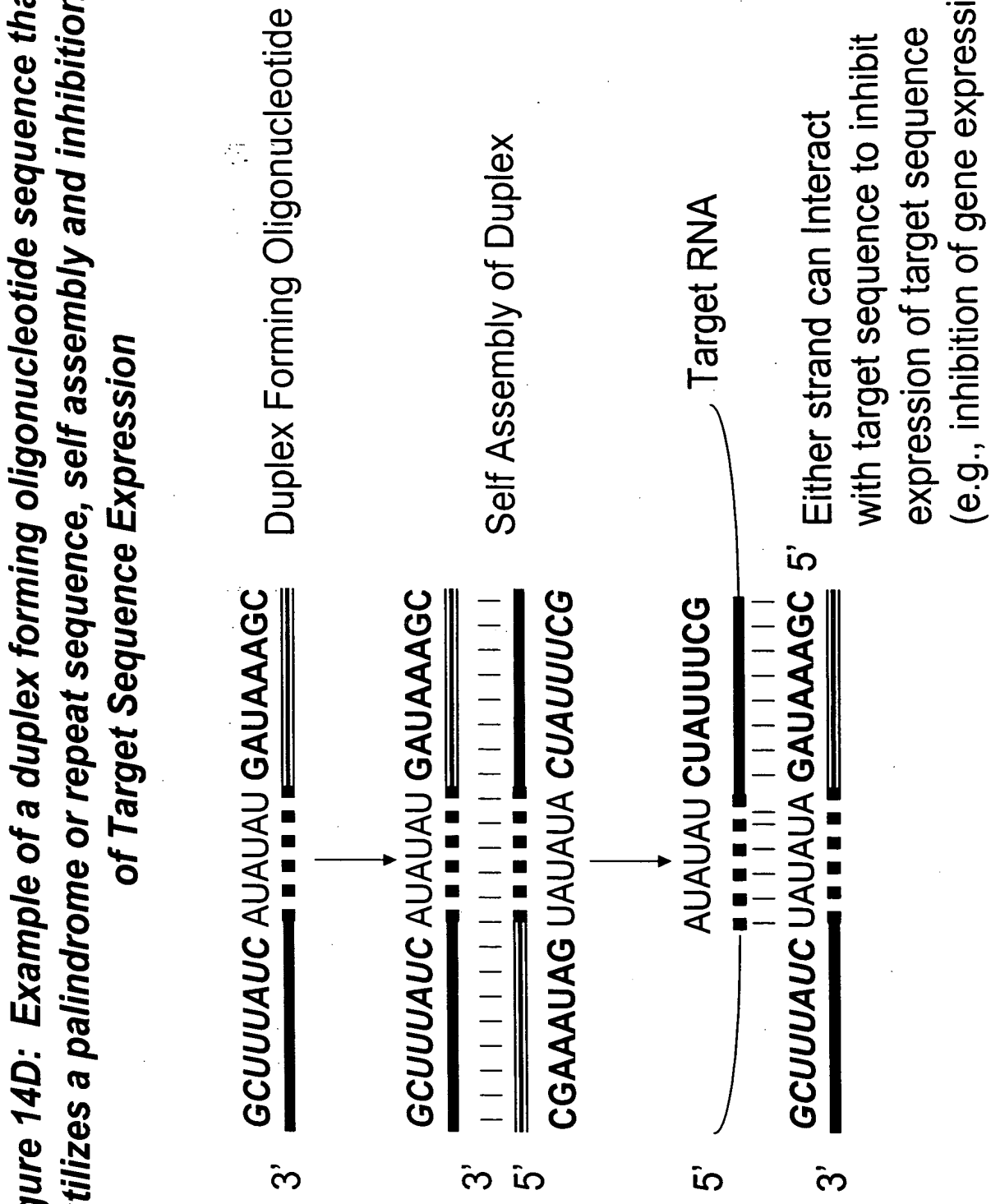


Figure 15: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences

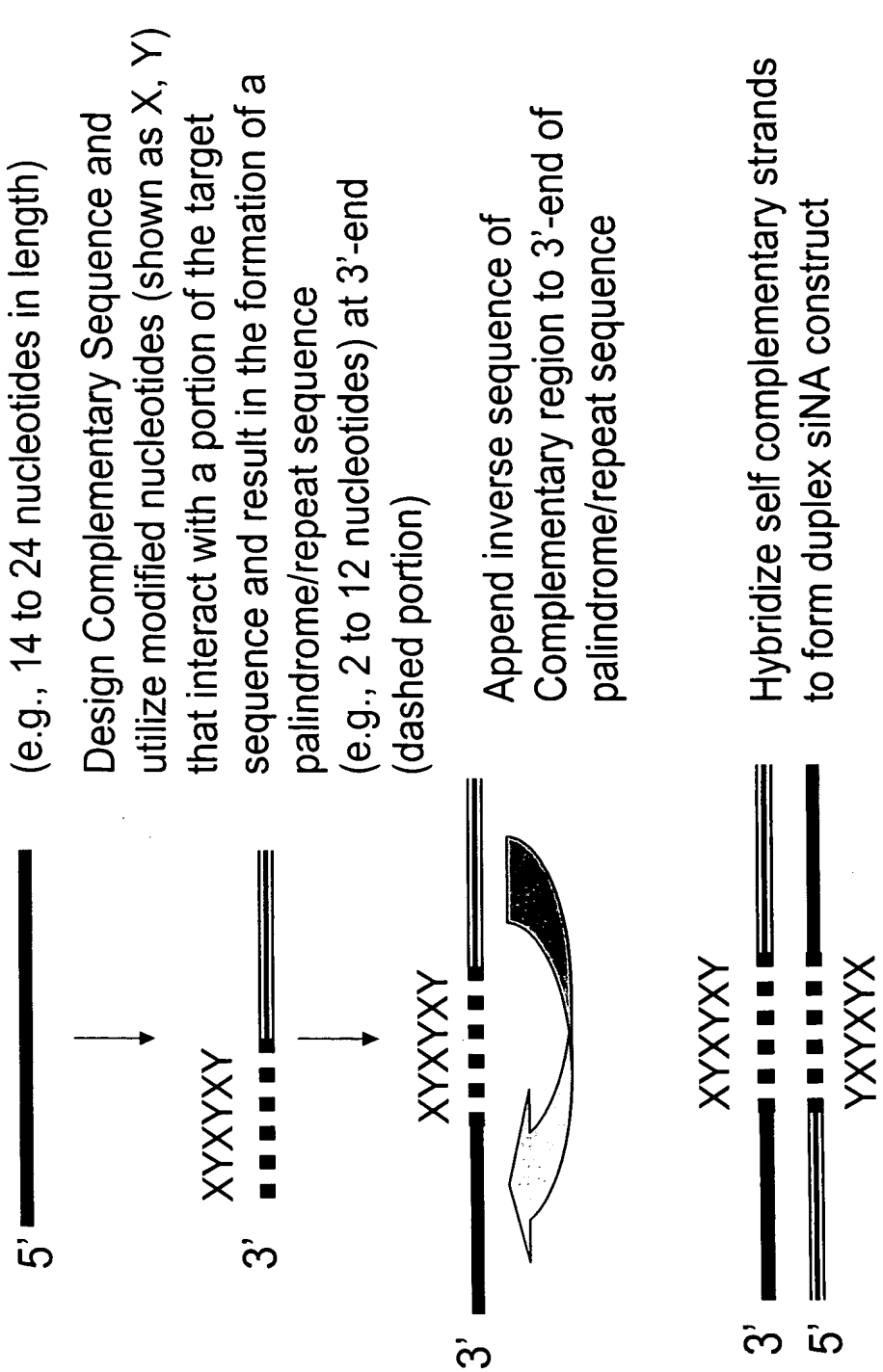


Figure 16: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

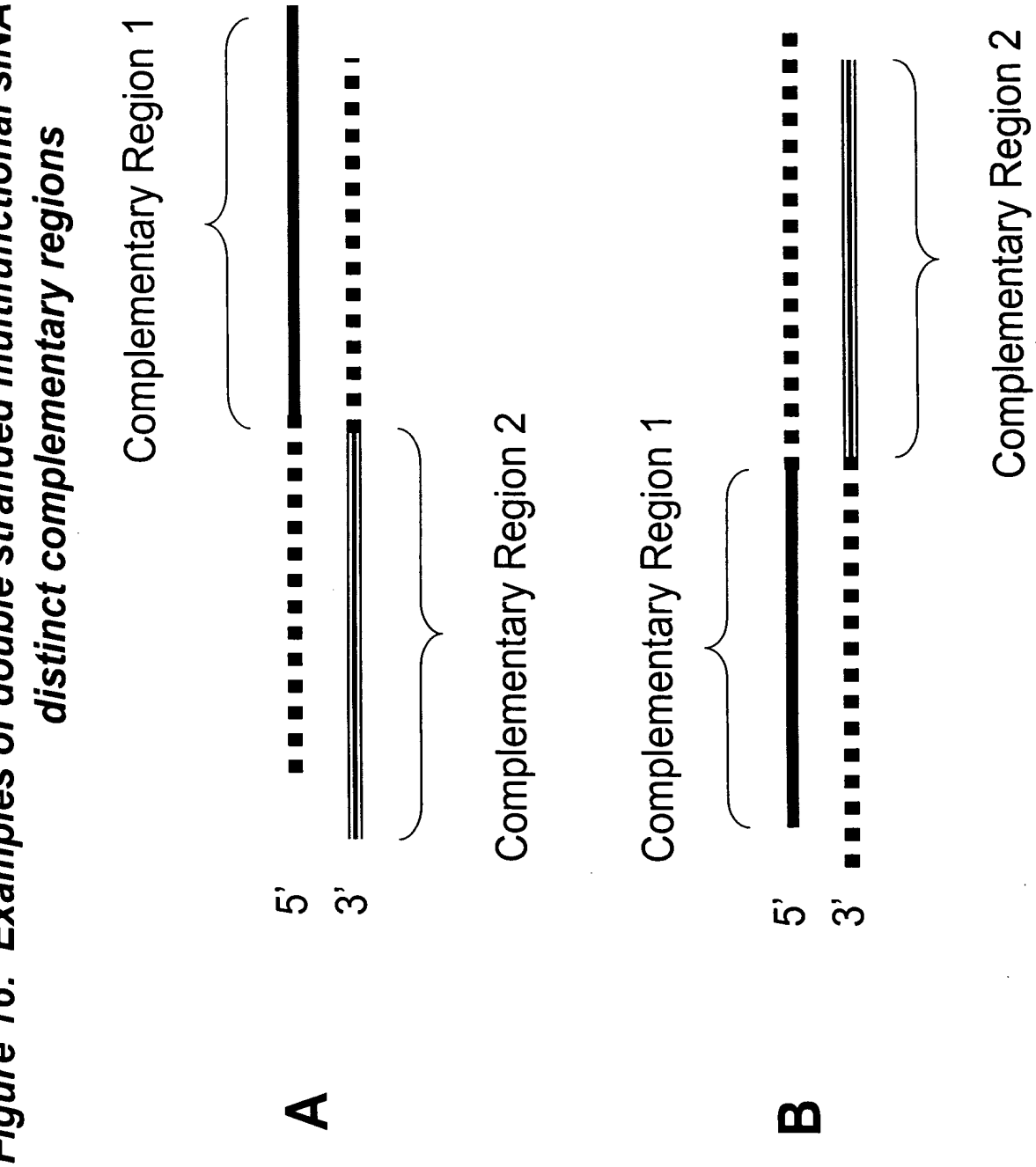


Figure 17: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

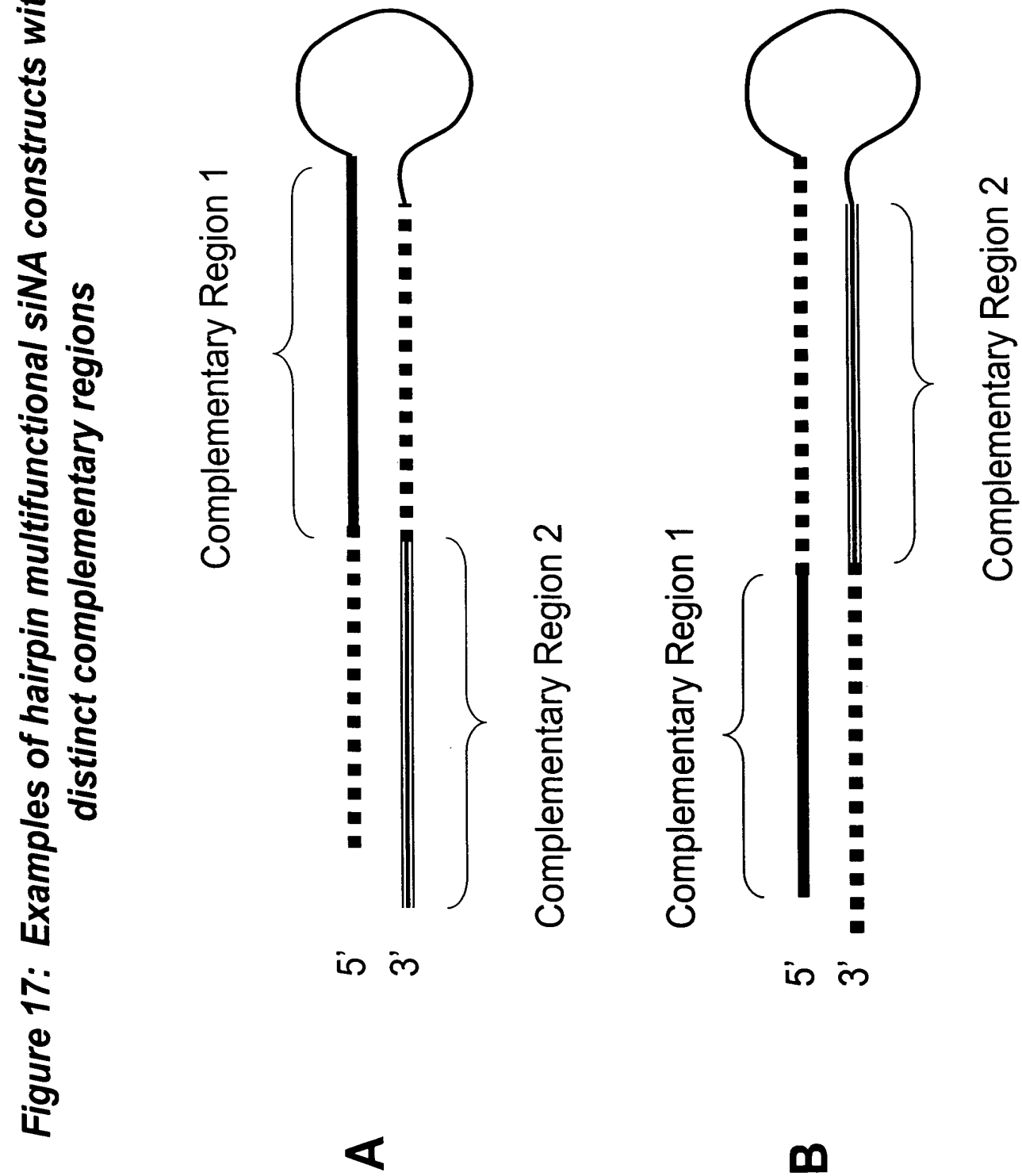


Figure 18: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

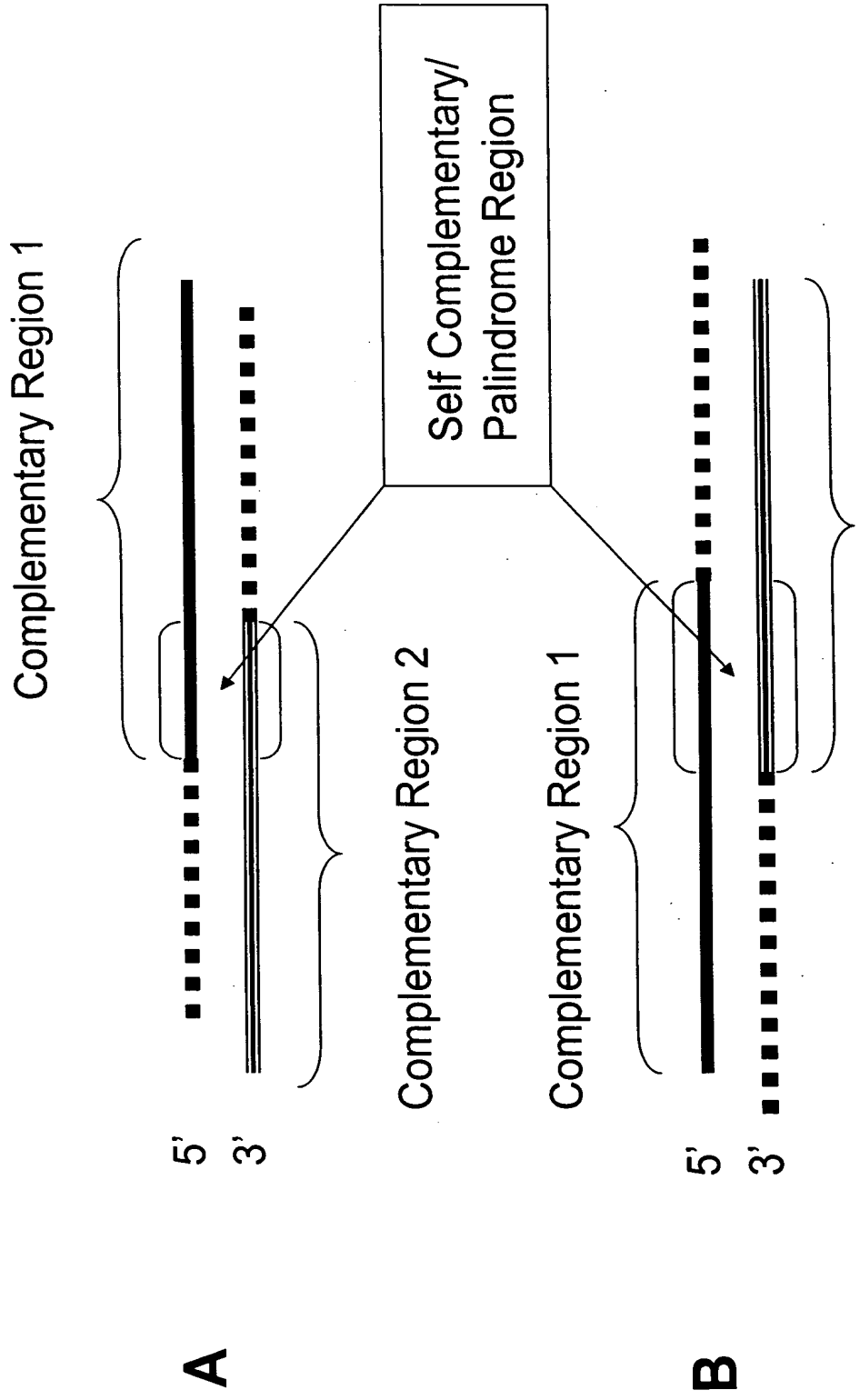
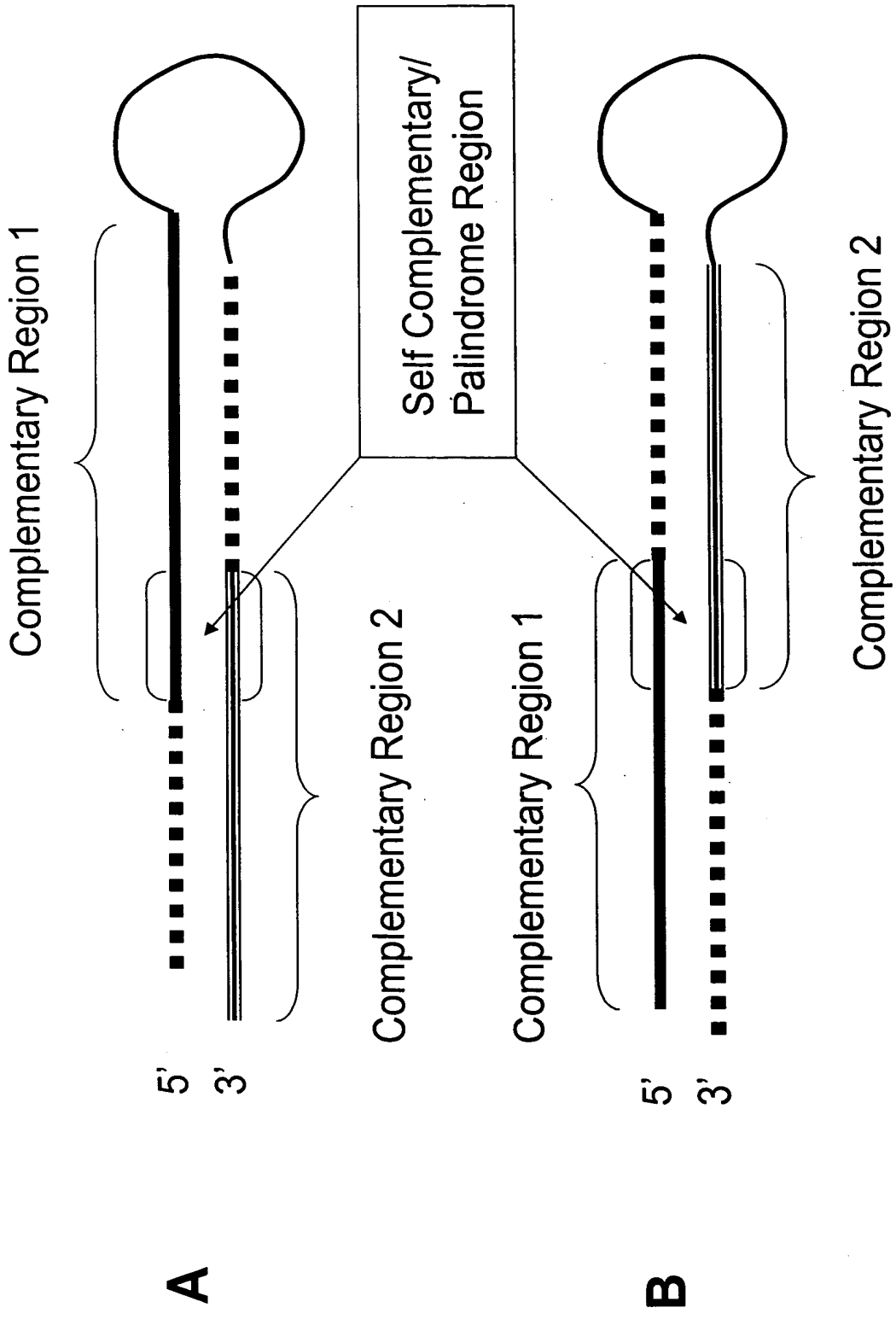


Figure 19: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



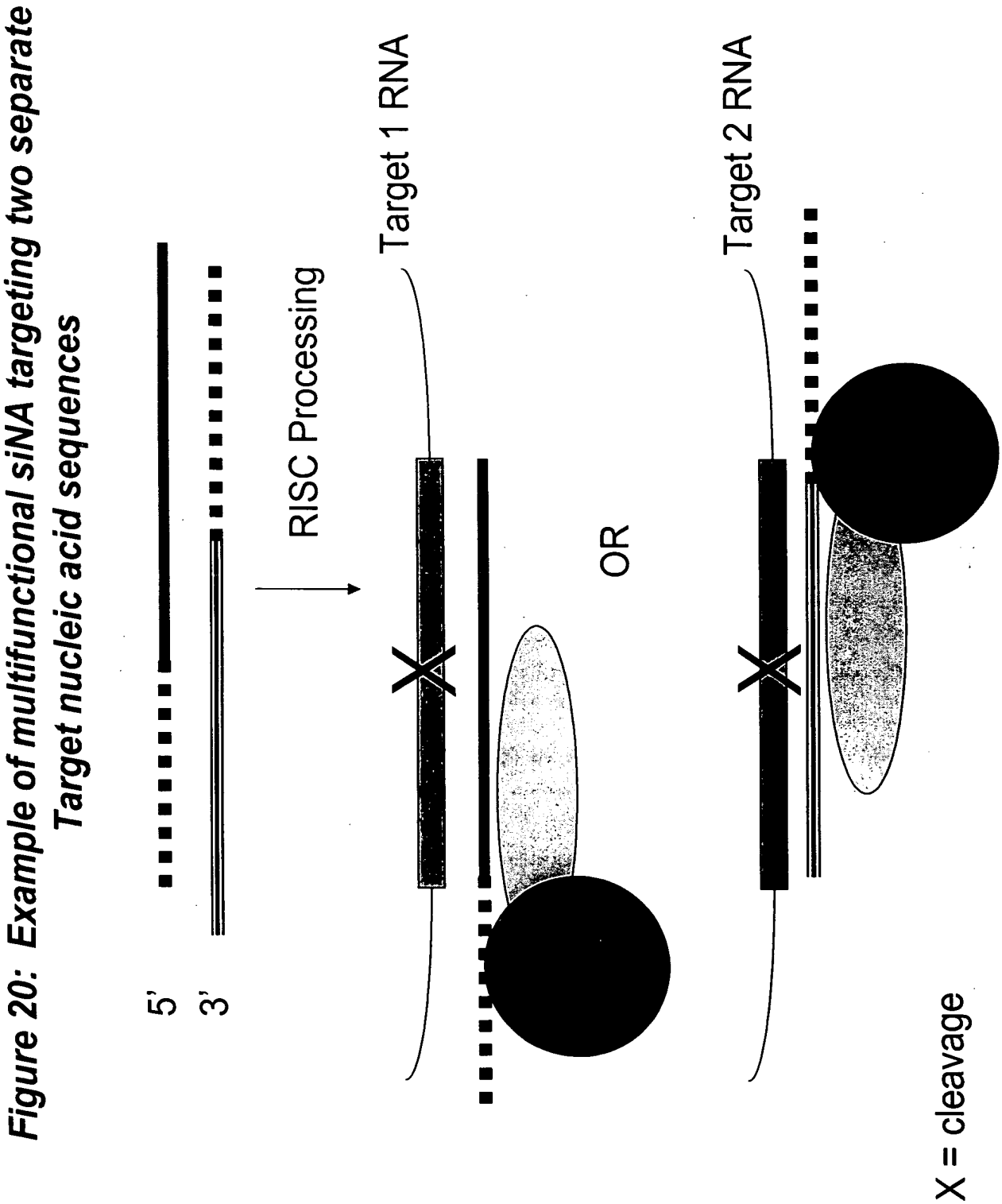


Figure 20: Example of multifunctional siNA targeting two separate Target nucleic acid sequences

Figure 21: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence